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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/536,502	12/14/2005	Chris D. Geddes	014835-101.02-029	6557

24239 7590 10/06/2010

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EXAMINER	
BERTAGNA, ANGELA MARIE	
ART UNIT	PAPER NUMBER
1637	
MAIL DATE	DELIVERY MODE
10/06/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/536,502	GEDDES ET AL.	
	Examiner	Art Unit	
	Angela M. Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 December 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5-10,12-16,18,20-22 and 24-27* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,5-10,12-16,18,20-22 and 24-27* is/are rejected.

7)☒ Claim(s) *8* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 23, 2009 has been entered.

Claims 1, 5-10, 12-16, 18, 20-22, and 24-27 are currently pending.

Claim Objections

2. Claim 8 is objected to because of the following informalities: The status identifier of this claim is incorrect.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out

the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1, 5-10, 12-16, 18, 20-22, and 24-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (WO 97/27317 A1; cited previously) in view of Lakowicz et al. (Photonics Spectra (October 2001) 35(10): 96, 97, 99-102, 104; cited previously) and further in view of Cao et al. (Journal of the American Chemical Society (July 2001) 123: 7961-7962; cited previously) and further in view of Qi *et al.* (Applied and Environmental Microbiology (2001) 67(8): 3720-3727; cited previously).

These claims are drawn to a method for detecting *Bacillus anthracis* in a sample. The method comprises hybridizing a sample suspected of containing *Bacillus anthracis* nucleic acids to an oligonucleotide immobilized on a layer of immobilized metal particles followed by hybridizing a fluorescently labeled oligonucleotide to the hybridized duplex.

Lockhart teaches methods of detecting target nucleic acids via array hybridization (see abstract and page 3, line 5 - page 6, line 12).

Regarding claims 1 and 16, the method of Lockhart comprises:

(a) providing surface-immobilized capture nucleotide sequence probe complementary to a first portion of a target nucleic acid (see page 71, lines 1-14; see also Figures 12 and 13)

(b) contacting a sample and the capture nucleotide sequence probe, thereby binding any target nucleic acids that are complementary to the capture nucleotide sequence probe (page 71, lines 1-14 and Figures 12-13)

(c) contacting any bound target nucleic acids with a free nucleotide sequence probe, wherein the free nucleotide sequence probe has an affinity for a second portion of the target nucleic acid and has a fluorophore attached thereto (see page 71, lines 21-28, page 72, lines 23-31, and Figures 12-13; pages 36-39 teach that the label may be a fluorophore)

(d) identifying the target nucleic acid by fluorescence emission resulting from excitation of the fluorophore following irradiation (see page 73, lines 9-14 and pages 69-70).

Regarding claims 6, 7, 21, and 22, Lockhart teaches detecting fluorescence emission with a device comprising a fluorescent scanner (pages 69-70).

Regarding claim 8, Lockhart teaches covalent immobilization of the capture nucleotide sequence probe to the surface (page 8, lines 20-23).

Regarding claim 9, Lockhart teaches binding the capture and free nucleotide sequence probes to the target nucleic acid under highly stringent hybridization conditions (pages 66-67).

Regarding claims 12, 14, 25, and 27, Lockhart teaches the use of a fluorophore having a low quantum yield, specifically rose bengal (see page 38, line 21).

Regarding claims 13 and 26, Lockhart teaches the use of fluorophores that can undergo two-photon excitation (see page 37, line 2, where fluorescein and rhodamine are taught).

Lockhart does not teach that the immobilized capture probes are attached to metal particles or a metal layer on a substrate as required by claims 1 and 16, respectively. Also, Lockhart does not teach that the free nucleotide sequence probe further comprises a metal colloid attached thereto for sandwiching the fluorophore between the metal colloid and the metalized substrate as required by claims 15 and 16. Lockhart also does not teach detection of *Bacillus anthracis* as required by claims 1 and 18.

Lakowicz teaches a method for increasing the fluorescence of a fluorophore using metal particles (page 96).

Regarding claims 1, 15, and 16, Lakowicz teaches that "Silver particles can have several beneficial effects on fluorophore brightness, suggesting improvements in applications such as DNA analysis (Figure 2)." Lakowicz teaches that the beneficial effects include increased photostability, decreased lifetime, increased quantum yield, and improved detectability (see Figure 2, page 96, column 1, and Table 1). Lakowicz also teaches that the intrinsic fluorescence from DNA and the extrinsic fluorescence from a fluorophore bound to DNA can be enhanced by sandwiching the fluorophore between metal particles (see Figures 5, 7, & 8 and pages 101-102). Lakowicz also teaches that the distance between the fluorophore and the metal particle should be between about 50 and about 200 Angstroms (see page 99, column 2). This range overlaps with the claimed range of about 50 Angstroms to about 500 Angstroms. Lakowicz further states, "There will be a zone near the surface where the effects are maximal (page 99, column 2)."

Regarding claims 5 and 20, Lakowicz teaches the use of silver particles (see Figures 2-5, 7 and 8, for example).

Regarding claims 6, 7, 21, and 22, Lakowicz teaches detecting fluorescence emission using a detection device comprising a spectrometer (page 97 and Figure 3, for example).

Regarding claims 10 and 24, Lakowicz teaches irradiating the fluorophore using a single photon or a two-photon excitation means (pages 99, 102, and 103 and Table 1).

Regarding claims 12-14 and 25-27, Lakowicz teaches using a fluorophore with a low quantum yield, such as Rhodamine B or rose bengal (see Figure 3 and page 100).

Lakowicz does not teach immobilization of nucleic acids onto the metal particles as required by claims 1, 8 and 16. Lakowicz also does not teach detection of *Bacillus anthracis* nucleic acids.

Cao teaches a method for synthesizing oligonucleotides modified with silver particles covalently bound to the 5' or 3' terminus (page 7961 and Figure 2). Cao teaches that these oligonucleotides may be used in nucleic acid hybridization assays (see page 7962 and Figure 2).

Cao does not teach detection of *Bacillus anthracis* nucleic acids.

Qi teaches a method for detecting *Bacillus anthracis* in a sample using PCR. Regarding this pathogen, Qi states, "*Bacillus anthracis* is a causal agent of anthrax, a serious and often fatal infection of livestock and humans. It is considered one of the most effective biological weapons of mass destruction because of its highly pathogenic nature and spore-forming capability and has attracted attention due to its potential use as a biological warfare agent (page 3720, column 1)."

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Lakowicz to the method of Lockhart. Specifically, an ordinary artisan would have been motivated to immobilize the capture oligonucleotides on a layer of immobilized metal particles positioned on the surface of the array and additionally attach a metal colloid to the fluorophore-containing detection probe so as to sandwich the fluorophore between two metal layers, since Lakowicz taught that such sandwiching of low quantum yield fluorophores, such as rose bengal, results in increased photostability, decreased lifetime, increased quantum yield, and improved detectability (see above). An ordinary artisan would have had a reasonable expectation of success in covalently attaching the capture and detection probes of Lockhart to metal colloids, since Cao taught methods of covalently attaching silver

particles to oligonucleotides (page 7961). Finally, regarding the ranges set forth in claims 4 and 19, as noted in MPEP 2144.05, "In the case where the claimed ranges 'overlap or lie inside ranges disclosed by the prior art' a *prima facie* case of obviousness exists." *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Woodruff*, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990).

An ordinary artisan also would have been motivated to apply the method resulting from the combined teachings of Lockhart, Lakowicz, and Cao to the detection of any clinically relevant nucleic acid, such as nucleic acids from the pathogenic microorganism, *Bacillus anthracis*, which Qi taught is "one of the most effective biological weapons of mass destruction (page 3720, column 1)." An ordinary artisan would have had a reasonable expectation of success in designing capture and free nucleotide sequence probes to detect *Bacillus anthracis*, since Qi taught that the complete *Bacillus anthracis rpoB* gene sequence was publicly available and successfully designed nucleic acid primers and probes from this sequence (see pages 3722-3724). Thus, the methods of claims 1, 5-10, 12-16, 18, 20-22, and 24-27 are *prima facie* obvious in view of the combined teachings of Lockhart, Lakowicz, Cao, and Qi.

5. Claims 1, 5-10, 12-16, 18, 20-22, and 24-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cao *et al.* (Science (2002) 297: 1536-1540; cited previously) as evidenced by Malicka *et al.* (Biopolymers (2003) 72(2): 96-104; cited previously) and Lukomska *et al.* (Biochemical and Biophysical Research Communications (2005) 328: 78-84; cited previously) in view of Lakowicz (US 2002/0160400 A1; cited previously and hereafter "Lakowicz I") and

further in view of Lakowicz *et al.* (Biochemical and Biophysical Research Communications (2001) 286: 875-879; cited previously and hereafter "Lakowicz II").

These claims are drawn to a method for detecting *Bacillus anthracis* in a sample. The method comprises hybridizing a sample suspected of containing *Bacillus anthracis* nucleic acids to an oligonucleotide immobilized on a layer of immobilized metal particles followed by hybridizing a fluorescently labeled oligonucleotide to the hybridized duplex.

Cao teaches a sandwich assay for detecting target nucleic acid from a pathogen in a sample (see abstract and Scheme 1 on page 1537).

Regarding claims 1 and 16, the method of Cao comprises:

(a) providing surface-immobilized capture nucleotide sequence probe complementary to a first portion of a nucleotide sequence in the pathogen (see Scheme 1 and page 1537, column 1, paragraph 2)

(b) contacting the sample and the capture nucleotide sequence probe, thereby binding any pathogen nucleic acids that are complementary to the capture nucleotide sequence probe (see Scheme 1 and page 1537, column 1, paragraph 2)

(c) contacting any bound pathogen nucleic acids with a free nucleotide sequence probe, wherein the free nucleotide sequence probe has an affinity for a second portion of the pathogen nucleic acid and has a fluorophore attached thereto (see Scheme 1 and page 1537, column 1, paragraph 2)

(d) identifying the pathogen using surface enhanced Raman spectroscopy (see Scheme 1 and pages 1537-1538).

Further regarding claim 1 and also regarding claim 18, Cao teaches using the method to detect *Bacillus anthracis* (page 1538, column 1).

Regarding claim 8, Cao teaches covalent immobilization of the capture nucleotide sequence probe to the surface (see Scheme 1 and page 1537, column 1).

Regarding claim 9, Cao teaches binding the capture and free nucleotide sequence probes to the pathogen nucleic acid under highly stringent hybridization conditions (see page 1539).

Regarding claims 12 and 25, as evidenced by Malicka at page 100, column 1, the Cy3 fluorophore taught by Cao has a low quantum yield.

Regarding claims 13 and 26, as evidenced by Lukomska at pages 78-80, the Cy3 fluorophore taught by Cao can undergo two-photon excitation.

Cao does not teach that the immobilized capture probes are immobilized to metal particles or a metal layer on a substrate as required by claims 1 and 16, respectively. Also, Cao teaches detection using Raman spectroscopy rather than fluorescence spectroscopy. Finally, Cao does not teach that the free nucleotide sequence probe further comprises a metal colloid attached thereto for sandwiching the fluorophore between the metal colloid and the metallized substrate as required by claims 15 and 16.

Lakowicz I teaches a method for increasing the fluorescence of a fluorophore using metal particles (see abstract and paragraph 13).

Regarding claims 1, 15, and 16, Lakowicz I teaches that the fluorescence intensity of a fluorophore conjugated to a biomolecule, such as DNA or RNA, can be increased at least 80 to 140 fold by positioning the fluorophore near a metal particle (paragraphs 13, 18, 71, and 84). Lakowicz I provides an example of this increase in fluorescence intensity in Figure 3, Figure 8,

Figure 19, and paragraphs 114-116, 122, and 131-132, where the intensity of a fluorophore is increased by sandwiching between silver island films. Lakowicz I further teaches that may be substituted for the silver island films (paragraph 71). Lakowicz I also teaches that the distance between the fluorophore and the metal particle should be optimized and separation distances between about 50 and about 2000 Angstroms, about 50 to about 200 Angstroms, and about 50 to about 300 Angstroms are particularly useful (paragraphs 71-72).

Regarding claims 5 and 20, Lakowicz I teaches the use of silver particles (paragraph 71) or gold particles (paragraph 70).

Regarding claims 6, 7, 21, and 22, Lakowicz I teaches detecting fluorescence emission using a detection device comprising a spectrometer (paragraph 76) or a fluorescent scanner (paragraph 91).

Regarding claim 8, Lakowicz I teaches covalent immobilization to the metal particles (paragraph 72).

Regarding claims 10 and 24, Lakowicz I teaches irradiating the fluorophore using a single photon (paragraph 149) or a two-photon excitation means (paragraphs 100 and 147).

Regarding claims 12-14 and 25-27, Lakowicz I teaches using a fluorophore with a low quantum yield, such as Rhodamine B, rose bengal, or fluorescein isothiocyanate (paragraphs 64, 66, and 84). Lakowicz I teaches fluorophores with a low quantum yield only fluoresce when they are adjacent to a metal particle (paragraph 105). Lakowicz I further teaches that these fluorophores can undergo two-photon excitation (paragraph 147).

Lakowicz II teaches that the intrinsic fluorescence from DNA and the extrinsic fluorescence from a fluorophore conjugated to a DNA molecule can be enhanced by

sandwiching the fluorophore between metal particles (see abstract, page 875, and page 877, and Figure 3). Lakowicz further teaches that the fluorescence enhancement in the presence of metal particles is analogous to surface-enhanced Raman spectroscopy (page 878).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Lakowicz I and Lakowicz II to the method of Cao. Specifically, an ordinary artisan would have been motivated to sandwich the Cy3 fluorophore between metal particles, such as a metal colloid, and measure fluorescence emission from the fluorophore, as taught by Lakowicz I, since Lakowicz I and Lakowicz II taught that the fluorescence signal of a low quantum yield fluorophore could be enhanced by sandwiching the fluorophore between metal particles (see above). Since Lakowicz II taught that fluorescence enhancement by metal particles was analogous to surface-enhanced Raman spectroscopy (page 878) and since the methods of Lakowicz I and Lakowicz II were directed to enhancing the fluorescence of an extrinsic fluorophore conjugated to a nucleic acid (see above), an ordinary artisan would have been motivated to utilize either of these analogous detection methods to detect *Bacillus anthracis* in the method of Cao with a reasonable expectation of success. Thus, the methods of claims 1, 5-10, 12-16, 18, 20-22, and 24-27 are *prima facie* obvious over Cao as evidenced by Malicka and Lukomska in view of Lakowicz I and further in view of Lakowicz II.

Response to Arguments

6. Applicant's arguments filed on December 23, 2009 have been fully considered, but they were not persuasive.

Rejection of claims 1, 5-10, 12-16, 18, 20-22, and 24-27 under 35 U.S.C. 103(a) based on the combined teachings of Lockhart, Lakowicz, Cao, and Qi

Regarding the rejection of claims 1, 5-10, 12-16, 18, 20-22, and 24-27 as being unpatentable in view of the combined teachings of Lockhart, Lakowicz, Cao, and Qi, Applicant first argues that the combined teachings of the cited references do not teach or suggest the claimed invention. In particular, Applicant argues that Lockhart in Figures 12-13 only describes an array having a large number of arbitrarily selected probes and does not teach or contemplate the requirement for specific probes as required by the claimed methods (see pages 9-10).

This argument was not persuasive, because, as discussed previously, Lockhart expressly teaches oligonucleotide capture probes that are immobilized on a solid support and complementary to a known sequence of a target nucleic acid (see, for example, page 9, line 21 - page 10, line 2, page 12, lines 3-15, page 20, lines 1-13, page 45, lines 7-30, and page 53, lines 21-30). Although Lockhart teaches at page 71 that different embodiments of the disclosed methods include the use of oligonucleotide probes that can be randomly selected, haphazardly selected, composition biased, inclusive of all possible oligonucleotides of a particular length, etc, the disclosed methods are not limited to these embodiments. Further regarding Applicat's arguments at pages 9-10, it is noted that the prior art is relevant for all that it contains or would have suggested to the ordinary artisan (MPEP 2123). In contrast to Applicant's arguments at page 9, the position of the Office did not change in the final rejection. Rather, the additional teachings cited were cited to rebut Applicant's specific arguments regarding the teachings of the Lockhart reference and demonstrate that the disclosure of the reference is more expansive than Applicant argues.

Applicant also argues that the labeled probes used in the method of Lockhart must include a ligatable oligonucleotide and a ligase (page 10). Applicant argues that this feature of the methods of Lockhart requires the free oligonucleotide probe to hybridize to the second portion of the target sequence at a position close enough to permit ligation and that the fluorescent label is located at the 5' end of the ligatable oligonucleotide probe (page 11). Applicant further argues that Lockhart does not recognize or discuss the importance of label placement in terms of its interaction with metallic particles on the substrate (page 11).

These arguments were not persuasive, because the importance of the label position relative to metallic particles on the substrate is discussed in the Lakowicz reference. As discussed above, an ordinary artisan would have been motivated by the teachings of Lakowicz to sandwich the fluorescent label used in the detection methods of Lockhart between metal particles in order to enhance the observed signal. It is further noted that the claimed methods recite open, "comprising" language, and therefore, do not exclude methods, such as the methods resulting from the combined teachings of the cited references, in which the free probe is ligated to the capture probe. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See In re Van Geuns, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). It is further noted that Lockhart expressly teaches that the position of the fluorescent label is not limited to the 5' terminus so long as the labeled probe can undergo a ligation reaction (see, for example, page 10, lines 3-13 and also pages 40 and 42). It is also noted that, contrary to Applicant's arguments at page 11, the free probes used in the methods of Lockhart are not limited to embodiments where all possible lengths of free probes are used. The methods of Lockhart also encompass the use of free probes of the same length.

Applicant also argues that the secondary reference (Lakowicz) does not teach or suggest placement of the fluorophore on the free probe as required by the rejected claims (see pages 11-12). In particular, Applicant argues that the teachings of Lakowicz are directed to increasing the intrinsic fluorescence of nucleic acids rather than increasing the fluorescence of external fluorophores (pages 11-12). Applicant also argues that the only discussion in the reference pertaining to external fluorophores contains negative teachings that would lead the ordinary artisan away from sandwiching an external fluorophore, such as those disclosed by Lockhart (pages 11-12).

These arguments were not persuasive, because the teachings of Lakowicz cited by Applicant as negative teachings only discuss complications in one specific embodiment involving the use of fluorescently labeled nucleotides, specifically exonuclease-based sequencing. As discussed previously, Lakowicz teaches that a limiting factor in exonuclease-based sequencing is the need to label each nucleotide after exonuclease catalyzed release from the target nucleic acid (see page 12 of the response, where the teachings of Lakowicz are reproduced). These teachings of Lakowicz do not appear to consider detecting external fluorescent labels to be problematic, but rather attaching external fluorescent labels to sequentially released nucleotides to be problematic. Since the methods of Lockhart are not directed to exonuclease-based sequencing and do not require the complicated post-cleavage labeling step required by exonuclease-based sequencing methods, the teachings of Lakowicz would not have lead the ordinary artisan away from using metallic particles as disclosed in Lakowicz to increase the signal of the external fluorophores covalently coupled to the disclosed free probes that are used in the methods disclosed by Lockhart. Attention is also directed to

MPEP 2145 and 2123, which state that a "teaching away" requires an active discouragement or disparagement of the proposed solution. It is also noted that the Lakowicz expressly suggests applying the disclosed methods of metal-induced fluorescence enhancement to nucleic acid hybridization assays conducted using external fluorescent labels (pages 102-103).

Applicant further argues that Lockhart only teaches the use of external fluorophores and does not teach detection of target nucleic acids based on intrinsic DNA fluorescence, and that Lakowicz discourages the use of a fluorophore and does not attach probe sequences to metal particles as required by the claims (pages 12-13). Applicant also argues that the combining the teachings of Lockhart and Lakowicz would render Lockhart unsuitable for its intended purpose (pages 12-13).

This argument was not persuasive, because as discussed above, the ordinary artisan would have been motivated by the teachings of Lakowicz to sandwich the external fluorophore contained in the free probe of Lockhart between metallic particles to enhance its signal. Doing so would not cause the method of Lockhart to depend on the detection of intrinsic DNA fluorescence and also would not render the method of Lockhart inoperable or unsuitable for its intended purpose. Rather, application of the teachings of Lakowicz to the method of Lockhart would have improved the method by increasing its sensitivity and enhancing the signal obtained from the external fluorophore contained in the free probe. It is also noted that the teachings of Lakowicz clearly indicate that metallic particles can be used to enhance the fluorescence properties of external fluorophores, such as rose bengal and rhodamine B (see Figures 3-4 on page 97). The teachings of Lakowicz also suggest suitable distances for placement of the fluorophore to maximize metal-induced enhancement of the fluorescence (page 98). It would

have been well within the capabilities of the ordinary artisan to apply these teachings of Lakowicz to the methods taught by Lockhart, since all that would be required is selection of a suitable external fluorophore and placement of the fluororphore at a suitable distance from the surface of the array. Since Lakowicz provided explicit guidance as to suitable external fluorophores and optimal metal-fluorophore distances, the ordinary artisan would have had a reasonable expectation of success in using metallic particles to enhance the fluorescence of the external fluorophores used in the methods disclosed by Lockhart. Furthermore, in contrast to Applicant's arguments, the fluorophores taught by Lockhart are not required to be located at the terminus of the free probes. Lockhart does not impose such a limitation on the probes, and the ordinary artisan would have recognized that the location of the fluorophore could be altered (e.g. to an internal position on the free probe of Lockhart), if necessary to provide a suitable distance between the fluorophore and metallic particles. The only requirement imposed on the probes by Lockhart is that they are able to undergo ligation (page 10, lines 3-13). Since the synthesis of oligonucleotide probes having internal fluorescent labels was routine in the art (see, for example, pages 40 and 42 of Lockhart), an ordinary artisan would have had a reasonable expectation of success in modifying the position of the external fluorophore contained in the free probes of Lockhart if necessary to apply the teachings of Lakowicz to the utmost advantage.

Finally, Applicant argues that the teachings of the additional secondary references (Cao and Qi) do not remedy the deficiencies of the Lockhart and Lakowicz references (pages 13-14). This argument was not persuasive, because these references are only relied upon to establish that the ordinary artisan would have had a reasonable expectation of success in attaching

oligonucleotides to silver particles and to provide motivation for detecting *B. anthracis* nucleic acids, respectively.

Since Applicant's arguments were not persuasive, the rejection has been maintained.

Rejection of claims 1, 5-10, 12-16, 18, 20-22, and 24-27 under 35 U.S.C. 103(a) as based on the combined teachings of Cao, Lakowicz I, and Lakowicz II

Regarding the rejection of claims 1, 5-10, 12-16, 18, 20-22, and 24-27 under 35 U.S.C. 103(a) as being unpatentable over Cao as evidenced by Malicka and Lukomska in view of Lakowicz I and further in view of Lakowicz II, Applicant argues that the combined teachings of the cited references do not render the claimed methods obvious. In particular, Applicant first argues that the Cao reference teaches away from the claimed methods (page 14), and that there is no motivation to combine the teachings of Cao with those of the additional seconday references cited in the rejection (pages 14-16). Applicant also argues that the teachings of Lakowicz II do not remedy the deficiences of the combination of Cao and Lakowicz II (page 18).

This argument was not persuasive, because the motivation to combine the references has been clearly set forth in the rejection. As discussed previously, the ordinary artisan would have been motivated to sandwich the Cy3 fluorophore used in the method of Cao between metal particles, such as a metal colloid, and measure fluorescence emission from the fluorophore, as taught by Lakowicz I, since Lakowicz I and Lakowicz II taught that the fluorescence signal of a low quantum yield fluorophore could be enhanced by sandwiching the fluorophore between metal particles (see above). Since Lakowicz II taught that fluorescence enhancement by metal particles was analogous to surface-enhanced Raman spectroscopy (page 878) and since the

methods of Lakowicz I and Lakowicz II were directed to enhancing the fluorescence of an extrinsic fluorophore conjugated to a nucleic acid (see above), an ordinary artisan would have been motivated to utilize either of these analogous detection methods to detect Bacillus anthracis in the method of Cao with a reasonable expectation of success. It is also noted that combination of the cited references does not render Cao unsuitable for its intended purpose or change the principle of operation of the method disclosed by Cao as argued by Applicant (see page 17). Rather, application of the teachings of Lakowicz I and II to the hybridization-based B. anthracis detection method disclosed by Cao would result in an improved hybridization-based B. anthracis detection method having increased sensitivity due to the metal-induced enhancement of the fluorescence of the Cy3 fluorophore used in the method of Cao. Substituting metal-enhanced fluorescence detection as taught by Lakowicz I and II for the SERS detection taught by Cao would also not change the principle of operation of the method of Cao, since the method suggested by the combined teachings of the cited references would still utilize a sandwich hybridization assay and an observable optical signal to detect the presence of *Bacillus anthracis* in a sample. It is further noted that the teachings of Cao do not constitute a teaching away from the proposed modification as argued by Applicant, because the combined teachings of Lakowicz I and II would have indicated to the ordinary artisan that the potential difficulties related to the use of external fluorophores described by Cao at page 1536 would have been obviated by sandwiching the fluorophore between metal particles as suggested by the combined teachings of Lakowicz I and II.

Applicant also argues that the rejection does not consider the invention as a whole (pages 16-17). This argument was not persuasive, because the combined teachings suggest the claimed

invention as a whole. The claimed invention as a whole appears to be the use of metal particles to sandwich an external fluorophore placed on the free probe used in a sandwich hybridization assay to detect the presence of *Bacillus anthracis* in a sample. The prior art of Cao teaches the use of a sandwich hybridization assay and SERS to detect the presence of Bacillus anthracis in a sample. Cao does not teach sandwiching the fluorophore between metal particles as required by the claims; however, the teachings of Lakowicz I and II would have suggested this modification to the ordinary artisan. Accordingly, the claimed invention as a whole is prima facie obvious in view of the combined teachings of the cited references.

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning (page 17), it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See In re McLaughlin, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In this case, as discussed in greater detail above, the rejection only relies upon the teachings present in the cited references and not Applicant's disclosure, and accordingly, the rejection is proper.

In response to applicant's argument that the examiner has combined an excessive number of references (pages 18-19), reliance on a large number of references in a rejection does not, without more, weigh against the obviousness of the claimed invention. See In re Gorman, 933 F.2d 982, 18 USPQ2d 1885 (Fed. Cir. 1991).

Finally, Applicant argues that the instant application was previously assigned to a different examiner, and that the present examiner has not given full faith and credit to the previous examiner's work as required by MPEP 704.01 (pages 19-20). Applicant's remarks concerning this point have been considered. However, it is noted that the claims have been amended since the time of the first action on the merits, and the present rejections have been made to properly address the claim amendments. Such action is not considered to be improper.

Since Applicant's arguments were not persuasive, the rejection has been maintained.

Conclusion

10. No claims are currently allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela M. Bertagna whose telephone number is (571)272-8291. The examiner can normally be reached on M-F, 9- 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Angela M. Bertagna/
Examiner, Art Unit 1637